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Possible Mechanism of GRP (Gastrin-Releasing Peptide)-Containing Nerves Regulating the Mucosal Microcirculation in the Rat Gastric Body

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Summary. **GRP (gastrin-releasing peptide)-containing nerves are most densely distributed in the gastric body throughout the gut. We previously found a marked decrease of GRP-immunoreactivity from nerve fibers in the oxyntic mucosa with stress-induced ulcers. The unusual release of GRP from the nerves under stress conditions seems to cause acute mucosal erosions. Since GRP has an action of vasoconstriction which may be responsible for the genesis of ulcers, the present study focuses on the relationship of GRP nerves and blood vessels.**

The GRP-immunopositive nerves were found to run close to the blood vessels throughout the mucosa. Scanning and transmission electron microscope observations showed that the nerve bundles containing GRP fibers were topographically associated with capillaries, but not with any glandular cells. The oxyntic mucosa is supplied exclusively by capillaries without smooth muscles which may respond to GRP. However, the capillaries here were accompanied by pericytes which reportedly are contractile in nature. Pericytes were frequently found intervening between the capillaries and nerves. The present finding suggests a possibility that GRP released from nerves reduces mucosal blood flow via the contraction of pericytes, finally causing mucosal ischemia and damage.

INTRODUCTION

The mucosa of the gastrointestinal tract is known to be heavily innervated by various types of peptidergic neurons. The gastric mucosa, especially the oxyntic mucosa, is characterized by a high concentration of nerves immunoreactive for gastrin-releasing peptide (GRP).[3,4] GRP is a potent stimulant of gastric acid release in the dog, cat, and human.[12,14] This effect, however, has been considered to be an indirect action via gastrin release from antral G cells.[14,26] It remains unsettled why the GRP-positive nerves are densely, rather more densely than in the antrum, distributed in the oxyntic mucosa, which contains none of the G cells. The concentrated distribution of GRP-immunoreactive nerves in the oxyntic mucosa supports a possible direct effect upon the acid-secreting area.

Recently, we found a remarkable decrease in the number of GRP-immunoreactive nerves in the oxyntic mucosa of rats with stress-induced ulcers, and we indicated that GRP is selectively depleted from nerves in the oxyntic mucosa under stress conditions.[7,13] Thus we suggested a possibility that GRP released in the fundus might play an important role in acute hemorrhagic erosions, although the involvement of GRP in ulceration has remained unknown.

In the course of our studies, we found an intimate relationship of GRP-immunoreactive nerves with blood vessels.[6] Nerve fibers distributed in blood vessels are usually believed to be vasoactive in nature. This morphological finding apparently is enigmatic because the oxyntic mucosa is supplied exclusively by capillaries, and arterioles are restricted to the bottom of the mucosa.[18] Blood capillaries lack a smooth muscle layer which would respond to GRP. The mechanism of the GRP-nerve supply involved in the mucosal microcirculation in the rat oxyntic area has thus become the theme of the present paper. This problem reminds us of a comparable phenomenon of association of VIP-immunoreactive nerves and blood capillaries in intestinal villi and some other areas.[6]

The present study aims to elucidate in detail the topographical relationship of GRP-containing nerves

and blood vessels; the possible involvement of GRP in ulcer formation will be discussed.

MATERIALS AND METHODS

Male Wistar rats weighing about 200 g were used in this study. Before sacrifice, the animals were fasted for 24 h.

Five animals under anesthesia by pentobarbital were perfused first with a physiological saline and then with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, and finally with Berlin blue-colored 5% gelatin solution which was kept at about 40℃. After the rats were cooled in a refrigerator, the stomach was removed and immersed in the same fixative for 6 h at room temperature. Cryostat sections about 20 μm thick were processed to the ABC (avidin-biotin complex) method by use of an anti-GRP serum (R 6902) diluted at 1:4,000. The anti-GRP serum was raised in a rabbit by using synthetic porcine GRP conjugated with bovine serum albumin as the antigen.[28] The site of the antigen-antibody reaction was revealed by a Biotin-StreptoAvidin Immunostaining Kit (BioGenex Lab, Doublin, U.S.A).

For conventional electron microscopy, five rats were perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The gastric body was dissected and immersed in the same fixative for 3 h. After postfixation in 1% osmium tetroxide for 2 h, they were dehydrated through a graded ethanol series and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and observed in a Hitachi H-7000 transmission electron microscope.

For scanning electron microscopy, three rats were perfused through the ascending aorta with Locke's solution followed by 2.0% glutaraldehyde in 0.1M phosphate buffer, pH 7.4. The gastric body was dissected and cut into small pieces about 2 mm in size and immersed in the same fixative overnight at room temperature. The tissue pieces were rinsed in phosphate buffer (pH 7.4) and treated with 6N NaOH for 20 min at 60℃, according to the NaOH maceration method.[21] Subsequently, the tissue blocks were dehydrated through a graded series of ethanol, transferred to isoamyl acetate, and critical point-dried by using liquid CO_2. The specimens were evaporation-coated with gold-palladium and examined by a Hitachi S-450 LB scanning electron microscope at an acceleration voltage of 10 kV.

RESULTS

Immunohistochemistry by use of the anti-GRP serum revealed a dense distribution of GRP-immunoreactive nerves in the mucosa of the gastric body (Fig. 1). The immunopositive nerve fibers of beaded structure ran perpendicularly between the fundic glands, frequently anastomosing into a ladder-like network throughout the lamina propria. Free endings of the nerve fibers were difficult to find. No topographic relationship was found between the GRP-immunoreactive fibers and specific cells constituting the fundic glands.

The GRP-immunoreactive nerves and blood capillaries could simultaneously be observed in single sections where the former were immunostained dark brown, while the latter were filled with blue gelatin (Figs. 2, 3). Both elements showed a marked tendency to be topographically associated. The GRP nerves were frequently shown to contact with the capillaries for a considerably long distance. The fibers tended to run gently winding, contacting and leaving the capillaries (Fig. 2). Occasionally, two GRP fibers were juxtaposed with a capillary on both its sides (Fig. 3).

Several arterioles could be recognized near the bottom of the mucosa. They revealed no close relationship to the GRP-immunoreactive nerves.

Transmission electron microscopy

Numerous nerve bundles consisting of several nonmyelinated fibers were found in the loose connective tissues between fundic glands. These bundles ran more closely to the blood vessels than to the glandular cells (Figs. 4-6). In the running course, the nerve fibers approached the blood vessels, leaving narrow spaces measuring only 150-200 nm (Figs. 5, 6). Blood vessels in the propria mucosae were exclusively capillaries which were fenestrated in type. Pericytes and their processes were frequently associated with these capillaries (Fig. 4). Pericytes showed comparatively electron-dense cytoplasm which contained a large number of filamentous structures.

Such a topographical proximity of nerve bundles towards blood vessels was not recognized towards the fundic glands. No distinct synapses and direct contacts were found between the nervous and glandular elements.

Individual nerve fibers showed typical varicosities. The swollen parts were filled with numerous small clear and large cored vesicles. Frequency of appearance of the large cored vesicles differed considerably

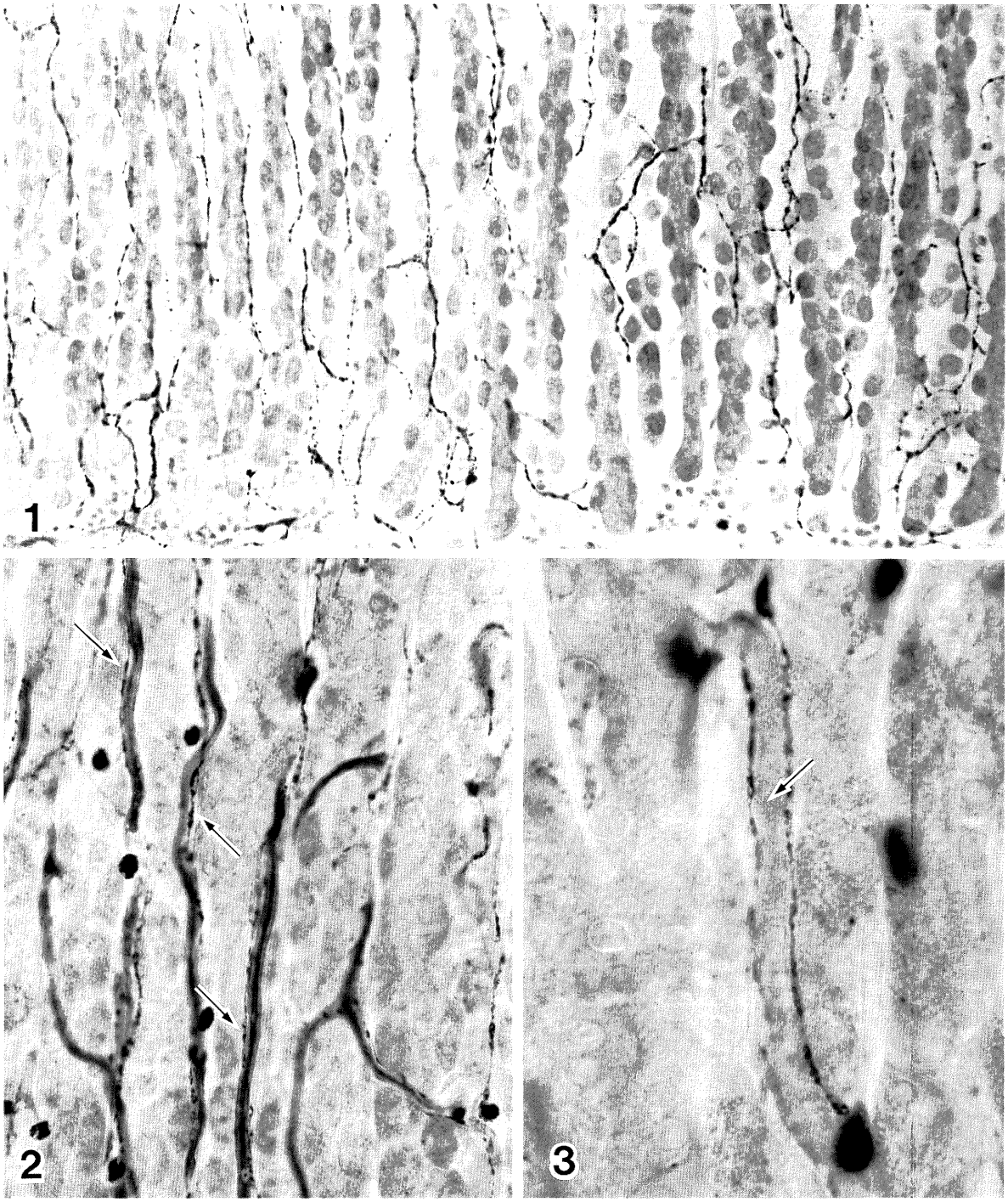


Fig. 1. GRP-immunoreactive nerves in the mucosa of rat gastric body. ABC immunostaining. Numerous beaded nerve fibers with GRP immunoreactivity are densely distributed throughout the propria mucosae. ×240
Figs. 2 and **3.** Micrographs showing the relationship between GRP-immunoreactive nerves and Berlin bule-perfused blood capillaries in the fundus region of the rat stomach. Note that beaded GRP fibers (*arrows*) are closely associated with capillaries. In Fig. 3, two GRP fibers contact with a capillary (*arrow*) on both its sides. Fig. 2: ×410, Fig. 3: ×1,000

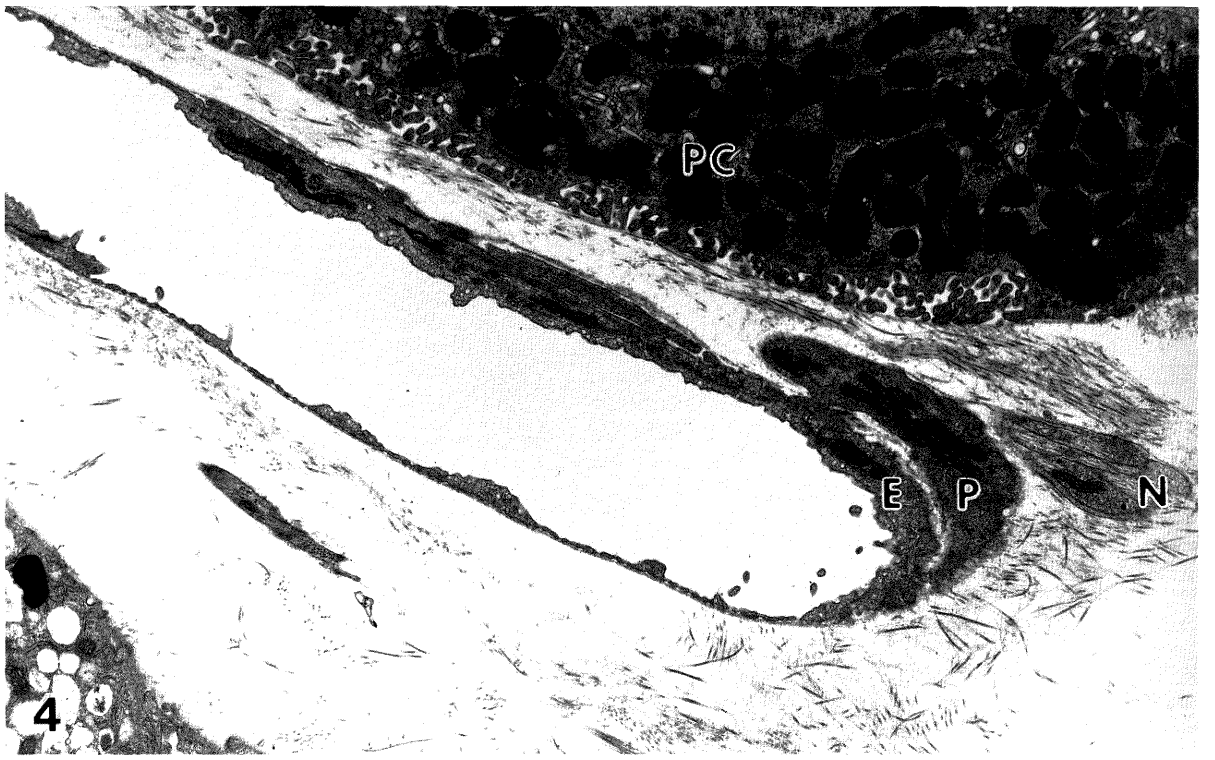
among nerves. Some of nerve fibers were, apparently, occupied by only the small clear vesicles (Fig. 6). The nerve fibers in a bundle were invested as a whole and compartmentalized into some fascicles by a Schwann sheath. The nerve fibers frequently and directly touched each other without an intervening Schwann sheath. It is noteworthy that the nerve fibers were only partly ensheathed by the Schwann sheath and partly exposed to the connective tissues, especially on the surface of the bundle.

Equally noteworthy was the finding that a pericyte often extended between the nerve and endothelium, thus causing a close contact of the nerve and pericyte.

Scanning electron microscopy

The topographically intimate relationship of the nerves and blood vessels was more evident by scanning electron microscopy (Figs. 7, 8). Essentially every blood vessel in the propria mucosae was confirmed to be a capillary in nature. The nerve bundles approached close to the capillaries and often coiled around them. The nerve bundles frequently issued a branch to the wall of the capillaries, which contributed to form a perivascular network but did not seem to end in a single termination (Fig. 7).

Pericytes of the blood capillaries had a slightly bulged fusiform cell body and long cytoplasmic processes extending along the capillary wall. In most of the pericytes, the cell body as well as its processes tended to extend along the long axis of the capillary. The pericytes were disposed separately from each other at varying distances, though rarely did they contact with each other via long processes. The main, or primary processes of the pericytes issued finer, secondary processes. These were bilaterally extended processes incompletely circling the capillary wall, usually surrounding only half or less of the circumference of the vessel. The ends of the processes branched up in dendritic fashion, and each of them adhered to the endothelial tube (Fig. 8).

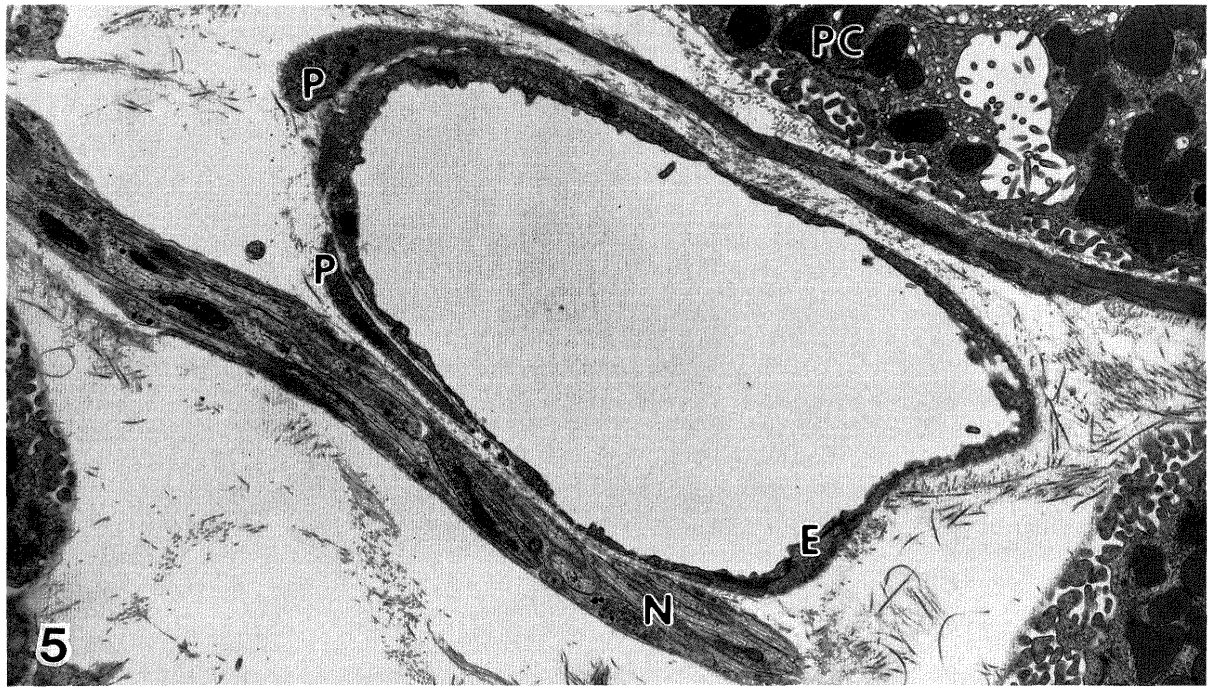


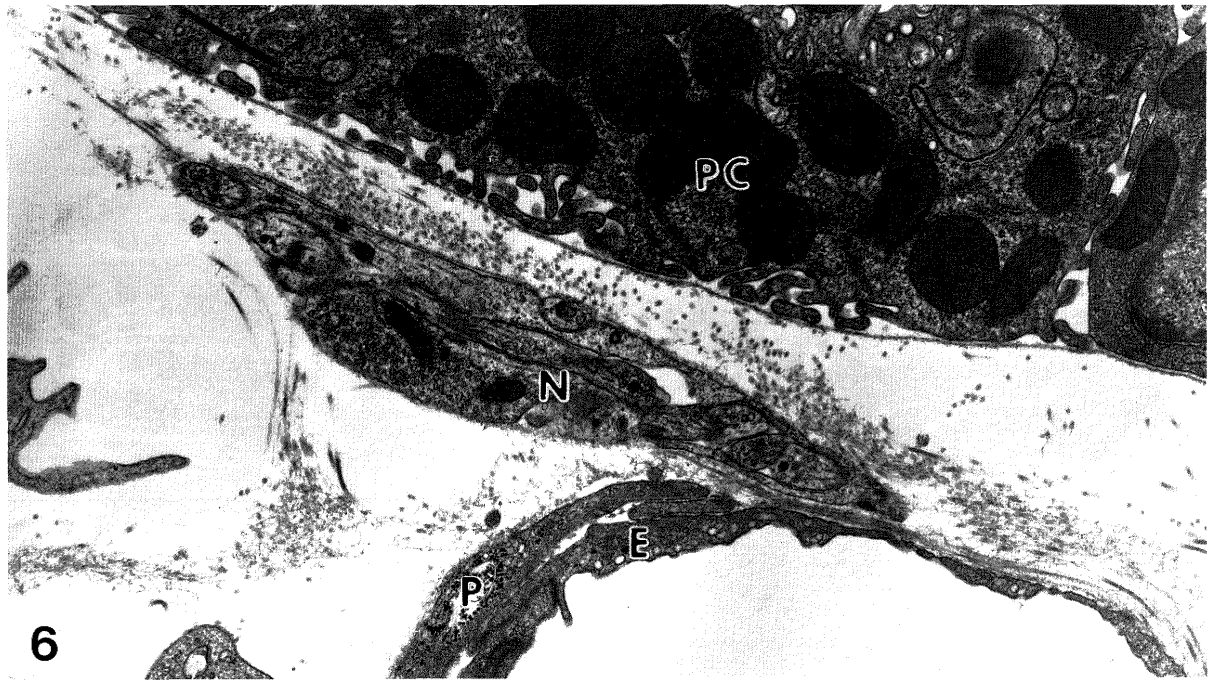
Figs. 4-6. Transmission electron micrographs showing the topographical relationship of blood capillaries and nerve bundles in the fundic mucosa of rats. A thicker or thinner process of a pericyte (*P*) intervenes between a capillary endothelium (*E*) and a nerve bundle (*N*). The nerve bundles (*N*) consisting of several nerve fibers approach fenestrated capillaries and their pericytes leaving only narrow spaces, but keep a considerable distance against glandular cells. Note that, in Fig. 6, nerve fibers filled with synaptic vesicles are exposed from their Schwann sheath, directly facing a pericyte. Fig. 4: ×8,500, Fig. 5: ×8,000, Fig. 6: ×16,400

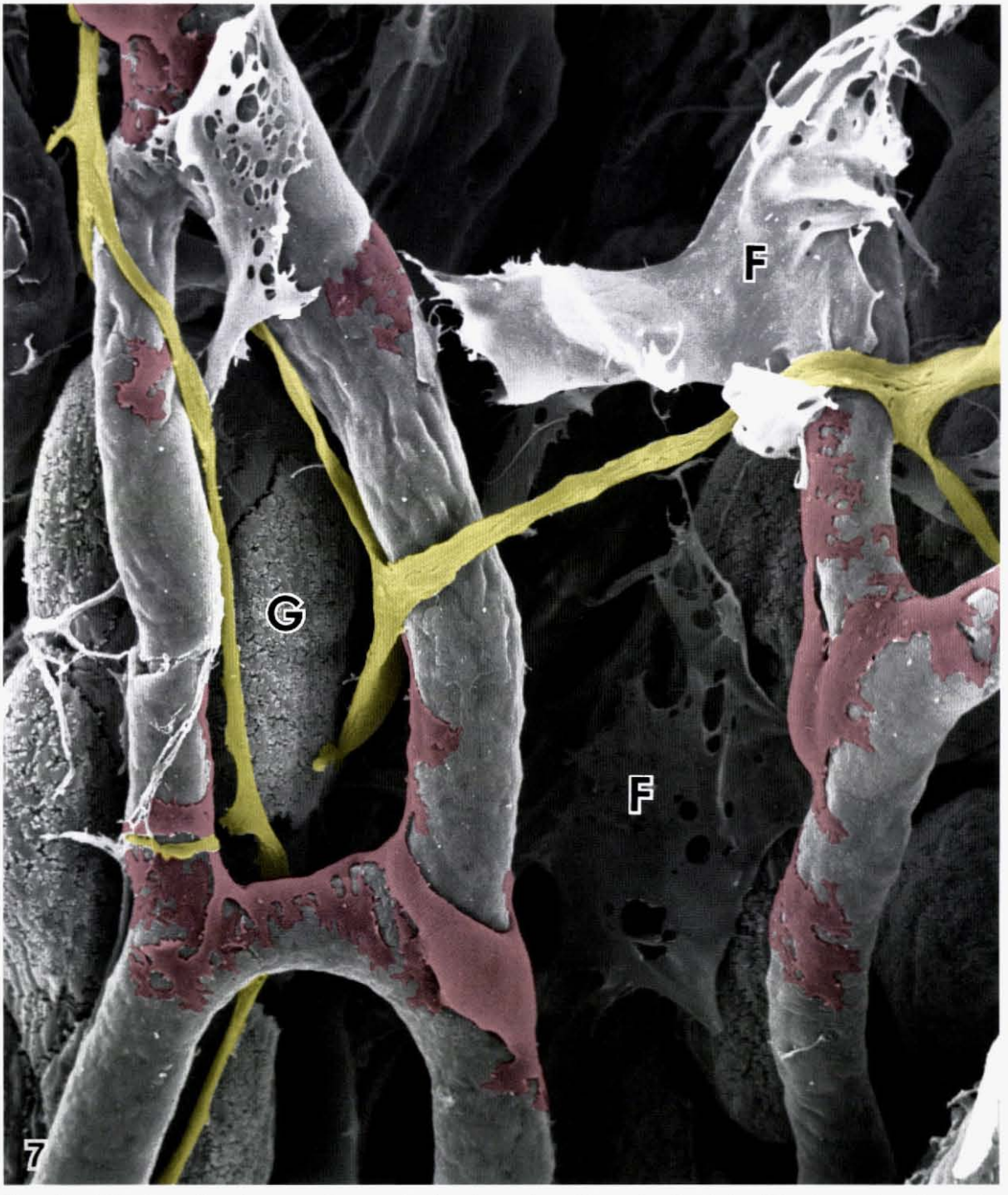
It was often recognizable that a nerve bundle ran for a long distance over a pericyte and its processes, apparently without any intervening elements except for the basement membranes which had been dissolved (Fig. 8).

DISCUSSION

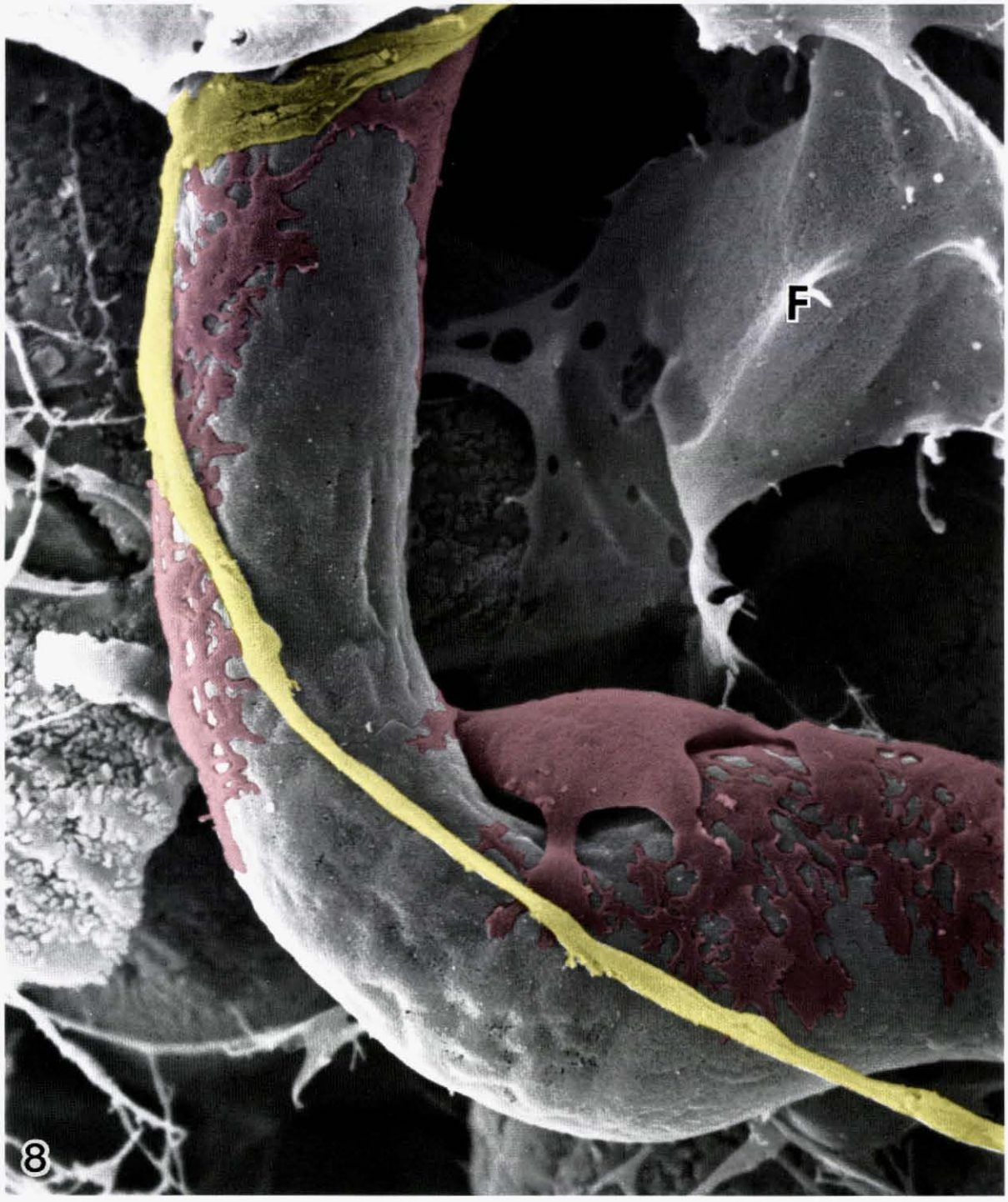
Our previous study revealed that the GRP-immunoreactive nerves which densely supply the rat oxyntic mucosa were conspicuously decreased in number under stress conditions; this change was ascribed to a severe release, even depletion, of GRP







Figs. 7 and **8.** Scanning electron micrographs showing a three-dimensional relationship of nerve bundles and capillaries in the fundic mucosa of rats. Nerve bundles (colored yellow) approach close to capillaries and partially coil around the capillary tubes which are associated with pericytes (colored red). *F*: fibroblasts; *G*: glandular cells Fig. 7: ×26,000, Fig. 8: ×6,400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from the nerves.[7,13] The release of GRP under stress conditions was supported by a radioimmunoassay study by MATSUBAYASHI et al.,[11] who demonstrated, in similarly stress-exposed rats, that plasma GRP levels elevated to about 2.5 times the normal level after exposure to stress for 5 h. We further suggested that the depletion of GRP is involved in ulceration by stress. This finding was made on the basis of our observations that the GRP depletion from the nerves was shown to always occur in association with the appearance of ulcers, both after stress and after vagal stimulation, and that vagotmy and certain anti-ulcer drugs were shown to prevent not only the development of stress ulcer, but also the depletion of GRP.[13]

Several pathogenetic factors have been considered to intervene in stress-induced ulcers. Among the possibilities are gastric hyperacidity, mucosal ischemia, bile reflux, decrease in mucosal energy metabolism, and deficient mucous barriers. Some workers have emphasized that a decrease in the mucosal blood flow may be a principal factor among these.[2,9,16] A significant decrease in mucosal blood volume in the stomach was observed in stress ulcers induced by thermal or head injury, hemorrhagic shock, and restraint plus water immersion.[15]

GRP is known to cause smooth muscle contraction and also vasoconstriction.[14] The present immunohistochemical staining performed in Berlin blue-perfused specimens found evidence for a close spatial relationship between the GRP-nerves and the blood vessels. Electron microscopic investigation of the GRP-immunoreactive fibers by means of the post-embedding technique indicated that the nerve fibers were closer to the blood vessels than to the glandular elements in the rat fundus (our unpublished data). Therefore, the possibility exists that GRP contracts the blood vessels supplying the oxyntic mucosa, causing local ischemia. The bottleneck in this hypothesis is that the blood vessels in the lamina propria of the oxyntic mucosa are capillaries in nature, devoid of any muscles which would respond to GRP.

The present electron microscopic observations showed existence of many pericytes on the capillaries in the oxyntic mucosa. The contractile ability of pericytes has been discussed since the first description by Rouget,[20] although there has been dispute concerning the identity of the "Rouget cells" in amphibia and the electron-microscopically recognized pericytes in higher vertebrates. Recent transmission and scanning electron microscopists are mostly of the view that the pericytes of capillaries are gradually transitional to the smooth muscles of arterioles and venules.[17,19,24] Further ultrastructural studies support the idea that the main function of pericytes may be the regulation of capillary flow by their contraction,[1,22,23,27] although most of the studies along this line have been carried out on the continuous (non-fenestrated) type of capillaries as in striated muscles, the heart, retina and central nervous system. Pericytes were shown histochemically to possess some contractile proteins, such as actin, myosin, and tropomyosin.[8,10,25]

In this respect the present finding seems worthy of attention that in the oxyntic mucosa pericytes intervene between the blood capillaries and the GRP-containing nerve bundles. An attempt to demonstrate that GRP directly causes contraction of the capillary system in the oxyntic mucosa is being made in our research group.

On the other hand, the existence of capillary-associated nerves supports the hypothesis that the nerves in question may release GRP and other active substances (probably at least acetylcholine[5] not only to vicinal target cells by paracrinia, but also into the blood by hemocrinia. Hemocrine release of GRP may partially account for the gastrin release in the stress condition. We previously pointed out the fact that GRP-immunoreactive nerves are fewer in number in the antrum where G cells are located and that they do not show any significant release of GRP after exposure to stress conditions.[13] At any rate, both the actions of GRP released in the lamina propria of the stomach and the significance of the peptide released into the general circulation must be targets of future explorations.

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